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Patent
Attorney's Docket No. 032425-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of Marshall S. Horwitz et al	)
Application No.: 09/132,231	) Group Art Unit: 1632
Filed: August 11, 1998	) Examiner: J. Brusca
For: METHOD FOR PRODUCING NOVEL DNA SEQUENCE WITH BIOLOGICAL ACTIVITY	)

DECLARATION PURSUANT TO 37 C.F.R. § 1.608

I, Phillip A. Patten, solemnly swear and attest to the truth of the following:

(1) I am employed as a Scientist at Maxygen Inc., and have been employed in this position for 4 years.

(2) I am a person of skill in the art of molecular biology, and particularly technology concerning evolutionary biotechnology, and have been quite familiar with this art for at least 18 years as evidenced by the curriculum vitae attached to my previous Declaration.

(3) I have read the Pieczenik patent (US 5,866,363), and am quite familiar with the technology discussed in it, particularly the evolution of antibody diversity and antigen-antibody recognition.

(4) I have been asked to clarify my position as set forth in my previous Declaration. In particular, I have been asked to discuss whether the screening of random epitope libraries expressed using lambda gt11 would have required "undue experimentation" as of the filing date of the original Pieczenik application (August 28, 1985).

(5) It has been explained to me that the determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. I understand that the test is not merely quantitative, and that a considerable amount of experimentation is permissible if such experimentation is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. It has been explained to me that the factors that are considered in determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

(6) As a result of my review of the Pieczenik patent and my knowledge of the state of art, I believe that using a lambda gt11 expression system to express a random peptide epitope library in order to identify naive antibodies that recognize a specific peptide would have required undue experimentation at the time that the Pieczenik application was filed in 1985. Although similar expression systems are commonly used to screen cloned DNA for genes expressing particular peptides or proteins using high affinity antibodies as probes, I fail to see how such a system could be employed for Pieczenik's methods as of his 1985 filing date. In particular, it would have been extremely difficult, if not impossible, to identify particular antibodies from a non-immunized animal that identify particular peptides within a randomly-generated population using a lytic phage expression system and the screening methods available in 1985.

(7) As I explained in my previous Declaration, it is known in the art that affinity maturation is a natural process of hypermutation and selection that occurs in vivo during

immunization and typically leads to an increase of one to several orders of magnitude in binding affinity for the immunizing antigen. The antisera from unimmunized mice described by Pieczenik would be expected to have low affinity for any antigens that they bind, as is characteristic of antibodies from unimmunized mice, making library screening very challenging. In fact, given that the naive antibodies isolated in Pieczenik would likely be polyclonal (recognize more than one antigen), the skilled artisan would reasonably see that it would require undue experimentation to identify a specific antibody/peptide cognate pair using the lambda gt11 expression system.

(8) I understand that the Examiner has alleged that the problem of antibodies cross-reacting with *E. coli* proteins could have been overcome by blocking or preadsorbing with *E. coli* extracts to remove these binding activities and that one skilled in the art would have known to introduce such a step in 1985. While such a step is routinely used in screening filters with high affinity antibodies, this would be problematic with libraries of random antibodies because of the expected low affinity and poor selectivity of binders in these libraries. The low affinity and selectivity would require that the artisan screen the libraries under very low stringency in order to allow these low affinity antibodies to bind stably enough to the expressed proteins to survive the washes and give rise to a more detectable chromogenic signal. The lower the wash stringency, however, the more background will come up. As the stringency is increased to reduce the background binding, the signal from naive antibodies binding to cognate peptides in the random library will correspondingly go down because of the low affinity of the antibodies. It is essentially a signal to noise ratio problem. The complex mix of *E. coli* proteins and whatever other proteins are used to block filters will create a lot of background as the stringency is decreased, making reproducible signals due to expressed random peptides undetectable. Certainly such signals would not be detectable without an extreme level of undue experimentation.

(9) As mentioned in my previous Declaration, the frequency of naive B cells that bind to an antigen with which the animal has not been immunized is about 10^{-5} to 10^{-6} (based on the measured frequency of antigen specific B cells in spleens). This low frequency alone make it such that screening random hybridomas for binders to particular antigens of interest would require an undue level of experimentation. Again, I am not aware of any research team in academia or industry that has screened 10^5 hybridomas for binders to an antigen of interest. In fact, 1000 is considered in the field to be a large screen.

(10) It has only been since the advent of very high complexity ($<10^9$) display libraries and powerful biopanning protocols such as phage display, i.e., with a filamentous phage vector, that it has been possible to obtain antibodies with moderate affinity (better than 100 nM) from naïve combinatorial libraries. Given that one of skill in the art can only now match a naive antibody with a random epitope using a phage display system, it would certainly have required undue experimentation in 1985 to identify such antibody interactions from among the high background generated in a lytic phage system.

(11) In summary, it would have required undue experimentation in August, 1985 to obtain, from naïve antibody libraries, antibodies against specific antigens of interest from within a random epitope library expressed using a lytic phage expression system.

(12) I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and

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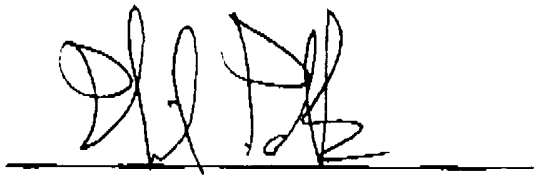
that such willful false statements may jeopardize the validity of the application and any patent issuing therefrom.

4-28-2000
Date



Signature